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Original Research

Risk Assessment Methodologies for Exposure of Great Horned Owls (*Bubo virginianus*) to PCBs on the Kalamazoo River, Michigan

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ABSTRACT

Dietary exposures of great horned owls (GHO; *Bubo virginianus*) to polychlorinated biphenyls (PCBs) in the terrestrial food web at the Kalamazoo River, Michigan, USA, were examined. Average potential daily doses (APDD) in GHO diets were 7- to 10-fold and 3-fold greater at the more contaminated location versus a reference location for site-specific exposures quantified as total PCBs and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$), respectively. Wetland/aquatic prey contributed significantly to PCB exposure and APDD. Estimates of risk based on comparison of modeled dietary intake (e.g., APDD) to toxicity reference values (TRVs), using a hazard quotient (HQ) methodology, varied between diet composition methods (mass basis vs numeric basis). Mass-basis compositions yielded greater HQs at all sites. Potential risks associated with dietary exposures ("bottom-up" risk assessment methodology) were less than (HQ $<$ 1) benchmarks for effects,. This result is consistent with risk estimates based on concentrations in tissues ("top-down" risk assessment methodology), and indicated PCBs posed no significant risk to terrestrial raptor species. Colocated and concurrent studies that evaluated GHO reproductive performance (nestling productivity) and relative abundance were consistent with results of the risk assessment. Measures of risk based on HQs were consistent with direct measures of ecologically relevant endpoints (reproductive fitness). Uncertainty in risk estimates is contributed during the selection of TRVs for effects in GHO based on $TEQ_{WHO\text{-}Avian}$ because of the absence of species-specific, dose-response thresholds. This evaluation indicated that a multiple-lines-of-evidence approach provided the best estimate of risk.

Keywords: Raptors Dietary exposure Bioaccumulation 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

INTRODUCTION

The great horned owl (GHO; *Bubo virginianus*) is a useful sentinel species for site-specific baseline ecological risk assessments at sites with large contiguous areas of contaminated environmental media. Their sensitivity to the toxic effects of some organic contaminants, such as organochlorine, organophosphate, and carbamate pesticides and their relatively great exposure as apex predators makes them valuable as a surrogate species for estimating risk to raptors in the terrestrial food chain (Sheffield 1997). Great horned owls have been used successfully in site-specific estimates of risk posed by polychlorinated biphenyls (PCBs) at the Kalamazoo River Superfund Site (KRSS) in Kalamazoo and Allegan Counties, Michigan, USA (Strause et al. 2007). This previous study utilized a "top-down" or "tissue-based" approach in which exposure was determined by measuring concentrations of PCBs in eggs and blood plasma of nestlings. The potential for risks from exposure to PCBs were assessed in a multiple-lines-of-evidence ecological risk assessment that included both comparing the measured concentrations of PCBs to toxicity reference values (TRVs) as well as concurrent measures of productivity and abundance in a "weight of evidence" to identify cause and effect linkages between the chemical stressor and any observed suboptimal population or community structure at the site (Fairbrother 2003).

A 2nd method for assessing potential risk to wildlife uses a predictive approach in which the exposure is inferred by measuring concentrations of the chemicals of concern (COCs), such as PCBs, in matrices other than the receptor species of interest. This predictive approach is often referred to as the "bottom-up" or "dietary-based" approach. In this method the exposure-response is predicted by use of food chain modeling, based on site-specific measures of concentrations of the COCs in wildlife food items or sediments or soils. In some cases, the actual dietary items can be identified via diet studies and quantified via forage base and food item sampling programs, while in other situations default or average values are used. Data specific to dietary composition and prey item COC concentrations are combined with receptor species' ingestion rate and body weight parameters

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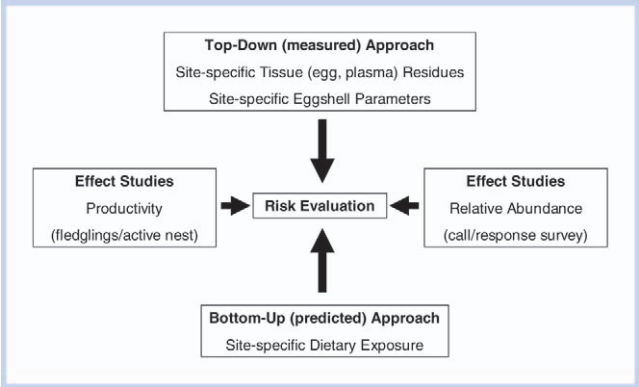

Figure 1. Multiple-lines-of-evidence used to assess risk to resident Kalamazoo River Superfund Site great horned owl (*Bubo virginianus*) populations.

to compute an average potential daily dose (APDD). This estimated daily dose is then compared to a dietary TRV to assess potential risks at the site. This study described the site-specific dietary exposure pathways to PCBs for GHOs at the KRSS. Site-specific dietary exposures (expressed as APDD) were then compared to TRVs for adverse effects determined in controlled laboratory studies to calculate hazard quotients (HQs) based on predicted exposure through diet. Additionally, the site-specific method of estimating dietary exposures was compared to a literature-derived APDD computed using diet composition values for an off-site GHO subpopulation residing in southeast Michigan (Craighead and Craighead 1956).

Due to limitations in the time and or resources available, few assessments apply both risk assessment methods simultaneously at the same location. Most assessments use the predictive method. While the 2 approaches are inherently linked, the accuracy and precision of the 2 methods are seldom compared. Therefore, the overall object of this study was to use a multiple-lines-of-evidence approach to evaluate the results of the predictive assessment approach with actual measurements of exposure (PCB concentrations in tissues, eggshell thickness) and population-level effects such as abundance or productivity at the same time at the same location (Figure 1). The 2 established methodologies of risk assessment (i.e., top-down vs bottom-up) were compared to determine how similar the predictions of risk would be based on both total PCBs, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) equivalents (TEQ) calculated from aryl hydrocarbon receptor-active PCBs. The equivalents are calculated by summing the products of concentrations of individual non-*ortho* (coplanar) (PCB 77, 81, 126, 169) and mono-*ortho* PCB congeners (PCB 105, 118, 156, 157, 167) and their respective World Health Organization (WHO) 2,3,7,8-TCDD toxic equivalency factors for avian receptors (Van den Berg et al. 1998). Avian-specific 2,3,7,8-TCDD toxic equivalents ($TEQ_{WHO\text{-}Avian}$) provide an estimate of potential risk from exposures to the most toxic PCB congeners.

Meeting these objectives required completion of the following:

1. Collection of GHO pellet and prey remains samples from active nest sites to identify dietary components and enumerate dietary composition;
2. Collection of representative prey item samples for the categories of prey (e.g., passerine birds, mice/voles) that contributed most significantly to GHO diet;
3. Determination of concentrations of PCBs and $TEQ_{WHO\text{-}Avian}$ based on congener-specific measurements;
4. Calculation and comparison of HQs based on total PCBs and total $TEQ_{WHO\text{-}Avian}$ between site-specific and literature-based diets;
5. Comparisons of bottom-up and top-down estimates of risk based on total PCBs and $TEQ_{WHO\text{-}Avian}$ using the HQ methodology; and
6. Incorporation of both the top-down and bottom-up approaches into a multiple-lines-of-evidence assessment that includes concurrent investigation of GHO relative abundance and reproductive productivity at each study site.

Additionally, information on the PCB/$TEQ_{WHO\text{-}Avian}$ concentrations between prey categories and food web sources (e.g., terrestrial vs. aquatic) was also assessed.

METHODS AND MATERIALS

Study sites

The KRSS includes 123 km of river extending from the city of Kalamazoo to Lake Michigan at Saugatuck, Michigan, USA. The primary COCs are PCBs, including total $TEQ_{WHO\text{-}Avian}$ from coplanar and mono-*ortho* PCB congeners. Polychlorinated biphenyls were used in the production of carbonless copy paper and paper inks for approximately 15 y (USEPA 1976). During this period, recycling of paper, including some carbonless copy paper resulted in releases of PCBs to the Kalamazoo River. The Kalamazoo River was placed on the Superfund National Priorities List in August 1990 due to the presence of elevated PCB concentrations in fish, sediments, and floodplain soils (BBL 1993).

Two sites within the KRSS were chosen for the GHO dietary study (Figure 2). These included the Fort Custer State Recreation Area (FC) and the former Trowbridge Impoundment (TB). Characterizations of these sites have been provided in earlier assessments of GHO exposure at the KRSS (Strause et al. 2007). The FC site is a reference area located approximately 7 km upstream of Morrow Pond Dam (the upstream limit of the KRSS) and 40 km upstream of TB. The FC site contains floodplain habitat similar to that present at TB and represents "current" regional background exposures in the watershed. The TB site is located in the Upper KRSS downstream of the point sources in the KRSS and it is 1 of 3 former impoundments in the Upper KRSS where removal of an in-stream dam to sill level has exposed former river sediments that are now heavily vegetated floodplain soils and riparian wetland habitat. The former TB impoundment includes the greatest area of contaminated soils (132 ha) and the greatest mean PCB soil concentrations (1.5×10^4 ng/g dry weight [dw]) in the river floodplain. The FC and TB sites were selected to make direct comparisons between GHO responses on a "high potential exposure" versus background "no elevated exposure" basis.

The GHO populations studied at each site were restricted to mated pairs occupying natural nests and artificial nesting platforms within the 100-y floodplain. The propensity for GHOs to use artificial nesting platforms allowed for better experimental control compared to wildlife studies that rely exclusively on natural nests. Nest platforms were placed throughout the FC and TB sites, and at TB the artificial platforms were placed to provide for a "worst-case exposure"

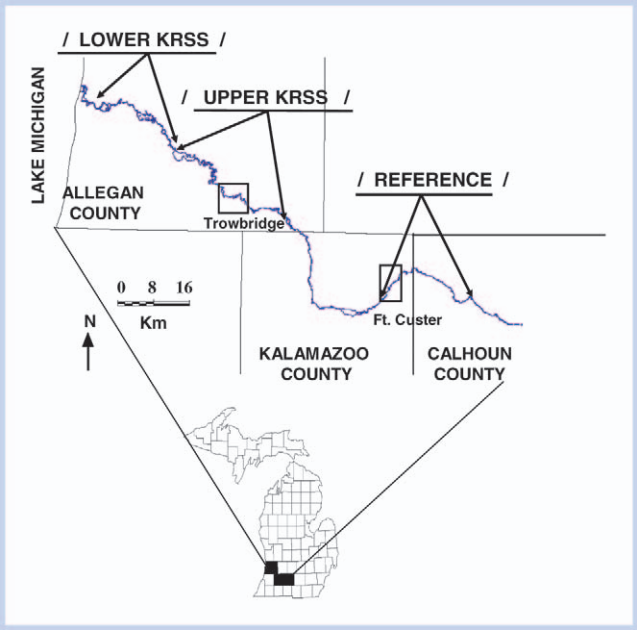

Figure 2. Kalamazoo River great horned owl (*Bubo virginianus*) study sites including the Reference sampling location (Ft. Custer), the Upper Kalamazoo River Superfund Site (Trowbridge), and Lower Kalamazoo River Superfund Site (LKRSS) sampling locations.

by maximizing GHO foraging in the most expansive areas of the contaminated floodplain.

Sample collection

The studies of GHO exposure to PCBs at the KRSS were part of a broader study to investigate PCB congener bioaccumulation and dynamics in the terrestrial and aquatic food webs of the Kalamazoo River floodplain that included representative samples from all tropic levels in resident terrestrial and aquatic communities (Millsap et al. 2004; Blankenship et al. 2005; Kay et al. 2005; Neigh, Zwiernik, Blankenship, et al. 2006; Neigh, Zwiernik, Bradley, Kay, Jones, et al. 2006; Neigh, Zwiernik, Bradley, Kay, Park, et al. 2006). All sample collections were completed within the 100-y floodplain. Representative taxa included raptors (owls and eagles), passerine birds, aquatic and terrestrial mammals, fish, terrestrial and aquatic invertebrates, plants and colocated soil, and sediment samples (Blankenship et al. 2005; Kay et al. 2005). The principal components of the GHO food chain in the KRSS floodplain are likely to include terrestrial mammals and terrestrial passerine or aquatic birds, although limited numbers of aquatic invertebrates also may be eaten (Figure 3).

Pellets and prey remains

Site-specific studies of GHO diet were completed only at active nest sites. Pellets and prey remains were collected to determine the principal prey items that comprised the diet of nestlings. Diet investigations were undertaken in conjunction with other GHO study objectives that included collection of blood plasma from nestling GHOs and monitoring of productivity (Strause et al. 2007). To minimize nest disturbances and to avoid bias this required that collection of pellets and prey remains were coordinated with these other investigations. Pellet and prey remains were collected from the nest, the base of the nest tree and beneath adult perch trees during the nestling blood sampling event. Additional samples were collected from the base of the nest tree and beneath feeding perches after the nestling GHOs fledged from the nest (2–3 weeks after blood was collected), and on 10-d intervals thereafter until no more samples could be collected. A final sample was collected from the nest and associated feeding perches during a "post-fledge" nest climb to clean and maintain the artificial nesting platform. Post-fledge climbs occurred between 4 and 10 weeks after the young had fledged. During each collection event, pellets and prey remains were systematically and completely removed from each location and packaged in a plastic jar as a composite sample representative of the specific collection site (e.g., nest, nest tree base, feeding/roosting perch). Samples were labeled to identify the nesting pair, dated and transported to the laboratory where they were exposed to naphthalene while drying to eliminate invertebrate scavengers. Prior to processing, pellet samples were sterilized by autoclave.

During each field season, all pellets and prey remains collected at an active nest were processed into 2 separate composite samples. Each composite sample was constructed around collections made during the 2 separate nest climbs completed at each active nest (nestling banding/plasma sample collection; post-fledge nest climb). Each composite included all collections from the ground that were completed concurrent with the nest climb and prey remains/pellet collections from the nest. Ground collections made subsequent to the banding event were combined into the 2nd (post-fledge nest climb) composite sample. This compositing scheme provided for reconciling the presence of large prey and bird remains among the ground and nest collections thus reducing the chance of overestimating the frequency of occurrence of large prey species because of their tendency to be represented in more than 1 pellet or prey sample (Marti 1974). The 2 distinct composite samples were processed independently and the prey items identified in each were summed to arrive at a final count for each nesting event.

Prey item identification/dietary composition

Relative proportions of prey items in the site-specific diet were determined by examining unconsumed prey remains (bones, fur, and feathers of animals too large to consume whole) as well as the skeletal remains in regurgitated pellets (Errington 1932, 1938; Hayward et al. 1993). All identifiable remains were sorted and quantified as to the minimum number of individuals from each taxon necessary to account for the assemblage of remains present in any given composite of samples. For mammalian prey items too large for owls to swallow whole (~100 g) and avian prey, the remains of the same prey item were frequently present in multiple samples. When this occurred, the items from within each discrete sampling event were examined together to reconcile the frequency of occurrence of larger prey and birds. Multiple prey item identification keys were utilized for comparative identification of mammalian and avian remains including owl pellet identification keys (Carolina Biological Supply, Burlington, NC, USA) and the vertebrate skeletal collection from the Michigan State University (MSU) museum. Avian remains (feathers) were identified with the aid of MSU Kellogg Biological Station bird sanctuary personnel. Prey items were identified to the best practical taxonomic classification and grouped by species/family and order into 7 prey categories relating to food web (aquatic vs terrestrial) and trophic level (primary vs secondary consumers) position.

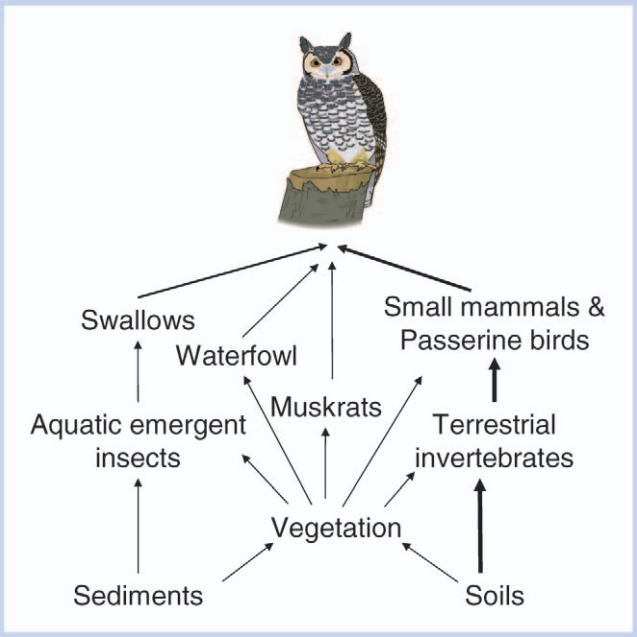

Figure 3. Great horned owl (*Bubo virginianus*) food chain and exposure pathways at the Kalamazoo River Superfund Site (KRSS).

These categories included: Passerine (terrestrial avian), waterfowl (aquatic avian), mice/vole (terrestrial primary consumers, small mammal), shrew (terrestrial secondary consumers, small mammal), muskrat (aquatic primary consumers, medium-size mammal), rabbit/squirrel (terrestrial primary consumers, medium-size mammal), and crayfish (detritivor/aquatic primary consumer, invertebrate).

The estimated dietary composition was based on the frequency of occurrence of all identifiable prey items and compiled on the basis of percent composition on a numeric basis (% number) and percent composition on a biomass basis (% biomass; Wink et al. 1987). Percent biomass was calculated by multiplying each identified prey item by the mean adult weight (male + female) for the particular species or family (Dunning 1984; Baker 1983). The small number of individual prey items that could not be positively identified to family or order was limited to unidentifiable parts of terrestrial birds and medium-size mammals. For biomass calculations, these items were assigned a mass value equal to the average mass computed for the representative species identified for that category at each respective nest site.

Prey collections for chemical analyses

Prey species represented in site-specific and literature-based GHO diets were collected from the FC and TB study sites and analyzed for total PCBs. Analyses of pellet and prey remains samples collected from FC and TB identified 6 general categories of GHO prey. These included passerine birds, waterfowl, mice/voles, shrews, muskrats, and rabbit/squirrel. A 7th category, crayfish, is represented in the literature-based diet and included in the diet analysis. Field sampling and processing methods for representative individuals from each of the 7 prey categories are described below.

Passerine birds collected from the FC and TB sites included the tree swallow (*Tachycineta bicolor*), house wren (*Troglodytes aedon*), and American robin (*Turdus migratorius*). A single European starling (*Sturnus vulgaris*) also was collected at TB. All live birds were collected at the end of the nesting period. Adult wrens and swallows were captured with mist nets or a trap-door mechanism. Additionally, dead individuals found at nest boxes were salvaged for analyses (Neigh, Zwiernik, Bradley, Kay, Jones, et al. 2006; Neigh, Zwiernik, Bradley, Kay, Park, et al. 2006). Adult robins were collected using pellet guns (MSU Aquatic Toxicology Laboratory, unpublished data). The starling (carcass) was recovered beneath an active GHO nest. Birds were promptly euthanized by cervical dislocation and carcasses were placed in solvent-rinsed sample jars and frozen at −20 °C. For chemical sample analysis, feathers, beaks, wings, legs, and stomach contents were removed and the whole body was homogenized in a solvent-rinsed grinder.

Waterfowl species sampled included merganser (*Mergus* spp.), mallard (*Anas platyrhynchos*), wood duck (*Aix sponsa*), and blue-winged teal (*Anas discors*). Waterfowl sampling was not included in the MSU Kalamazoo River food web investigations. Waterfowl samples used in this GHO diet exposure study were collected in August 1985 by the US Fish and Wildlife Service. The US Fish and Wildlife Service collected adult and immature ducks from 5 locations in the KRSS. Sampling locations included Morrow Pond and the Menasha and Trowbridge impoundments in the Upper KRSS, and the Allegan State Game Area and Saugatuck Lake downstream of Allegan Dam in the Lower KRSS. For chemical analysis the feathers, beaks, and entrails were removed and the remaining carcass was homogenized in a solvent-rinsed grinder (MDNR 1987).

Small mammals collected included mice (*Peromyscus* spp., *Zapus hudsonius*), voles (*Microtus pennsylvanicus*), shrews (*Sorex cinereus*, *Blarina brevicauda*), red squirrels (*Tamiasciurus hudsonicus*), and chipmunks (*Tamias striatus*). All small mammals were trapped using pit-fall or Sherman live traps placed alternately within a 30×30 m^2 sampling grid sited in the floodplain. Two sampling grids were located at FC and 4 grids were set up at TB (see figure 1 of Blankenship et al. [2005]). Captured species were sacrificed by cervical dislocation and carcasses were placed in solvent-rinsed sample jars and frozen at −20 °C. Prior to chemical analysis, stomach contents were removed and the remaining whole body (including pelage) was homogenized in a solvent-rinsed grinder (Blankenship et al. 2005).

Muskrats (*Ondatra zibethicus*) were collected along the riverbank throughout FC and TB using body-gripping "conibear" traps. Samples were frozen at −20 °C until processing for chemical analysis. Processing of whole-body samples included removal of the pelage, a coarse grind, and further homogenization in a commercial blender (Millsap et al. 2004).

Crayfish (*Cambarus* spp. and *Orconectes* spp.) were collected along the riverbank at FC and TB by use of wire minnow traps set adjacent to the small mammal sampling grids. For chemical analyses, the whole body was homogenized in a solvent-rinsed grinder (Millsap et al. 2004).

Chemical analysis—extraction/clean-up

Concentrations of PCB congeners were determined by use of US Environmental Protection Agency (USEPA) method 3540 (SW846). The details of the soxhlet extraction and sample preparation and clean-up have been described previously (Neigh, Zwiernik, Bradley, Kay, Park, et al. 2006). Prey items were homogenized with anhydrous sodium sulfate (EM Science, Gibbstown, NJ, USA) using a mortar and

pestle. All samples, blanks, and matrix spikes included PCB 30 and PCB 204 as surrogate standards (AccuStandard, New Haven, CT, USA). Extraction blanks were included with each set of samples. Quality assurance/quality control sets composed of similar tissues were included with each group of 20 samples. Concentrations of PCBs, including di- and mono-*ortho*-substituted congeners were determined by gas chromatography (Perkin Elmer AutoSystem, Perkin Elmer, Waltham, MA, USA; and Hewlett Packard 5890 series II, Hewlett Packard, Palo Alto, CA, USA) equipped with a ^{63}Ni electron capture detector (GC-ECD). Concentrations of coplanar PCB congeners were determined by gas chromatograph mass selective detector (GC-MS; Hewlett Packard 5890 series II gas chromatograph interfaced to a HP 5972 series detector). Polychlorinated biphenyls were reported on a mass wet weight (ww) basis. A solution containing 100 individual PCB congeners was used as a standard. Individual PCB congeners were identified by comparing sample peak retention times to those of the known standard, and congener concentrations were determined by comparing the peak area to that of the appropriate peak in the standard mixture. Di-and mono-*ortho*-substituted PCB congeners were detected by selected ion monitoring of the 2 most abundant ions of the molecular cluster and the limit of quantification was conservatively estimated (minimum surface to noise ratio of 10.0) to be 1.0 ng PCB/g ww, using an extraction mass of 20 g, a 25 pg/μL standard congener mix and 1-μL injection volume. For coplanar PCB congeners, method detection limits varied among samples but were maintained at $\leq$0.1 ng/g ww for all samples using the sample-specific extraction mass and a minimum surface to noise ratio of 3.0. TurboChrom (Perkin Elmer) was used to identify and integrate the peaks. Total concentrations of PCBs were calculated as the sum of all resolved PCB congeners. Total PCB concentrations in waterfowl samples collected by the US Fish and Wildlife Service were quantified as Aroclor 1260 (MDNR 1987).

TEQ computation

Concentrations of $TEQ_{WHO\text{-}Avian}$ in prey item tissues were calculated to produce the most conservative estimate of body burden concentrations originating from coplanar and mono-*ortho* PCB congeners. Polychlorinated-dibenzo-*p*-dioxins and polychlorinated-dibenzofurans were not measured and were not included in TEQ computation. Whenever a PCB congener was not detected, a proxy value equal to one-half the limit of quantification was multiplied by the toxic equivalency factor to calculate the congener-specific TEQs. Co-eluting congeners were evaluated separately. Polychlorinated biphenyl congener 105 frequently co-eluted with congener 132, congener 156 frequently co-eluted with 171 and 202, congener 157 co-eluted with congener 200, and congener 167 co-eluted with congener 128. In order to report the maximum $TEQ_{WHO\text{-}Avian}$, the entire concentration of the co-elution groups was assigned to the mono-*ortho* congener. Among the 6 GHO prey categories analyzed at the MSU Aquatic Toxicology Laboratory (excludes waterfowl samples), the maximum combined contributions to total $TEQ_{WHO\text{-}Avian}$ of congeners 105, 156, 157, and 167 was 4.2% (passerine), 46% (mice/vole), 5% (shrew), 1.2% (muskrat), <1% (rabbit/squirrel), and <1% (crayfish), respectively.

Individual non-*ortho* and mono-*ortho* PCB congener concentrations in waterfowl samples were estimated from the quantified Aroclor 1260 total PCB concentrations by multiplying the geometric mean total PCB concentration by a congener-specific fractional composition value (Schwartz et al. 1993). The greatest observed congener-specific fractional value (percent composition basis) determined among 4 technical Aroclor mixtures (1242, 1248, 1254, 1260) was selected to account for inherent differences in Aroclor batch production processes. Bioaccumulation factors of 10 and 3 were applied to PCB congeners 126 and 169 to account for the selective enrichment (weathering, metabolism) of these 2 congeners that was measured in bald eagle (*Haliaeetus leucocephalus*) (PCB 126) and lake trout (*Salvelinus namaycush*) (PCB 169) eggs analyzed as part of the Schwartz et al. (1993) study. Using this approach, the combined contribution of congeners 105, 156, 157, and 167 to total $TEQ_{WHO\text{-}Avian}$ in waterfowl was <1%.

Toxicity reference values

In this study, TRVs were used to evaluate the potential for adverse effects due to PCBs including $TEQ_{WHO\text{-}Avian}$. Ideally, TRVs are derived from chronic toxicity studies in which a total PCB or $TEQ_{WHO\text{-}Avian}$ dose–response relationship has been observed for ecologically relevant endpoints in the species of concern, or alternately in a wildlife species rather than a traditional laboratory species. Chronic studies should also include sensitive life stages to evaluate potential developmental and reproductive effects, and there must be minimal impact from co-contaminants on the measured effects.

Toxicity reference values used in this assessment were based on values reported in the literature for no observable adverse effect levels (NOAELs) and lowest observable adverse effect levels (LOAELs) for total PCBs and $TEQ_{WHO\text{-}Avian}$ (Table 1). The dietary PCB NOAEL for GHO was based on the controlled, laboratory study on the reproductive effects of PCBs on the screech owl (*Otus asio*; McLane and Hughes 1980). In that study, screech owls were fed a diet that contained 3 mg PCB/kg, ww. At this dose, no effects were observed on eggshell thickness, number of eggs laid, young hatched and fledged. A TRV for the GHO was estimated from the toxicity information available for the screech owl by use of allometric relationships, body weight and food consumption given by Sample et al. (1996). This resulted in a TRV for GHO, expressed as a daily dose, of 4.1×10^2 ng PCB/g bw/d. A LOAEL was not identified in that study so the LOAEL value of 1.23×10^3 ng PCBs/g body weight (bw)/d was estimated by applying a NOAEL to LOAEL uncertainty factor of 3. No additional uncertainty factors were applied to account for potential intertaxon variability, because the NOAEL is in the range determined for the chicken, the most sensitive bird species tested (Platonow and Reinhart 1973; Lillie et al. 1974).

No studies of the effects of $TEQ_{WHO\text{-}Avian}$ were available for deriving TRVs, and no studies were found in which there was a closely related test species to GHO. A subchronic laboratory study (10-week exposure period) by Nosek et al. (1992) found that intraperitoneal injections of 2,3,7,8-TCDD at concentrations of 1.0×10^3 pg TCDD/g/week (1.4×10^2 pg TCDD/g bw/d) caused a 64% decrease in fertility and a 100% increase in embryo mortality in ring-necked pheasants (*Phasianus colchicus*). This exposure concentration was used as the dietary TEQ-based LOAEL for GHO. A NOAEL was not directly available from this study and had to be derived from the limited dose data. Because effects due to the

Table 1. Toxicity reference values (TRVs) used to calculate hazard quotients for total polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) in great horned owl (*B. virginianus*) diet and eggs[a]

	Dietary-based TRVs (ng PCBs/g bw/d) (pg $TEQ_{WHO\text{-}Avian}$/g bw/d)		Tissue-based TRVs (ng PCBs/g) (pg $TEQ_{WHO\text{-}Avian}$/g)	
	NOAEL	LOAEL	NOAEC	LOAEC
Total PCBs[b]	410	1,230	7,000	21,000
$TEQ_{WHO\text{-}Avian}$	14[c]	140[c]	135[d]	400[d]

[a] NOAEL = no observable adverse effect level; LOAEL = lowest observable adverse effect level; NOAEC = no observable adverse effect concentration; LOAEC = lowest observable adverse effect concentration; bw = body weight.
[b] McLane and Hughes (1980).
[c] Nosek et al. (1992).
[d] Elliott et al. (1996, 2000) and Woodford et al. (1998).

exposure were pronounced in the test subjects, a safety factor of 10 was applied to derive a NOAEL of 1.4×10^1 pg TEQ/g bw/d. Limitations of the study include the evaluation of TCDD exposure and not PCB-TEQ exposure, differences in species, and use of injections of 2,3,7,8-TCDD in a subchronic study verses feeding 2,3,7,8-TCDD contaminated food in a chronic exposure study.

Because co-eluting congener contributions are included in some mono-*ortho* PCB congener concentrations used in this risk assessment the PCB-based TEQs may overestimate exposure relative to 2,3,7,8-TCDD. In this instance, the use of a TRV based on 2,3,7,8-TCDD exposure is likely to yield conservative estimates of risk when applied to PCB exposure. Also, tolerance to TEQ-based exposure by birds is species specific (Woodford et al. 1998), and the TRVs derived from Nosek et al. (1992) are likely to be protective of GHO because the *Galliformes* used in the study are among the more sensitive species to the effects of 2,3,7,8-TCDD (Hoffman et al. 1998). The available information indicates that raptors, such as the American kestrel (*Falco sparverius*), osprey (*Pandion haliaetus*), and bald eagle, are more tolerant than gallinaceous species to the effects of PCBs and TEQ (Elliott et al. 1996, 1997; Hoffman et al. 1998; Woodford et al. 1998). Thus, for GHO a more closely related raptorial species such as American kestrels would be the ideal basis for TRVs. However, in the few studies in which kestrels were exposed to PCBs, there was either inadequate dose–response information or incomplete assessment of ecologically relevant endpoints.

Tissue-specific TRVs (egg-basis) for total PCBs and $TEQ_{WHO\text{-}Avian}$ used in previous assessments of GHO exposure at the site (Strause et al. 2007) are included (Table 1). The egg-based TRVs are included to aid interpretation of the bottom-up and top-down methodology comparisons completed in this study.

Average potential daily dose (APDD)/risk assessment

The amount of PCBs ingested by GHOs was calculated using the wildlife dose equation for dietary exposures (USEPA 1993). The APDDs for total PCBs and $TEQ_{WHO\text{-}Avian}$ were calculated for GHOs using the site-specific diets for GHO determined in this study, and for comparison purposes a literature-based diet for a separate population of Michigan GHOs (Craighead and Craighead 1956). All APDDs were based on diets with prey composition compiled on a biomass basis (Equation 1). Average potential daily dose calculations also included the incidental ingestion of floodplain soils that could potentially be associated with GHO foraging activity.

$$\text{APDD} = \sum(C_k \times FR_k \times NIR_k) \quad (1)$$

C_k = Geometric mean and upper 95%CL concentration of total PCBs or $TEQ_{WHO\text{-}Avian}$, ww in the kth prey item category of GHO diet, or alternatively floodplain soils.

FR_k = Fraction of GHO diet (based on mass) represented by the kth prey item category.

NIR_k = Normalized GHO ingestion rate of the kth prey item (g prey/g bw/d, ww).

Concentrations of PCBs and $TEQ_{WHO\text{-}Avian}$ in representative prey items collected from the KRSS were determined using the methods described previously and are presented in the following section. FR_k (mass basis) was determined for the GHO subpopulation cohorts at both FC and TB, and from a previous study (literature-based diet) of GHO populations in southeast Michigan (Craighead and Craighead 1956). A conservative assumption for the value of FR_k is that GHO at the KRSS will obtain 100% of their diet requirements from the 100-y floodplain (site use factor = 1). NIR_k (0.056 g/g bw/d) was derived from daily ingestion rates and mean body weights reported for GHO (Craighead and Craighead 1956). Additional PCB and $TEQ_{WHO\text{-}Avian}$ dietary exposure from incidental soil ingestion was calculated for TB GHOs using both the site-specific and the literature-based dietary composition and geometric mean concentrations of PCBs measured for TB soils. Incidental soil ingestion contributions to dietary exposure were not calculated for the FC GHOs because of the very low concentrations of PCBs present in FC soils. Geometric mean and upper 95% confidence level (CL) geometric mean concentrations of total PCBs in TB floodplain soils (as an added protective measure nondetects were removed from the data set prior to computing mean and upper 95% CL values) were obtained from previous investigations at the site (BBL 1994). Concentrations of total PCBs in soils were considered to be 85% bioavailable and contain 65% moisture (estimated from Studier and Sevick [1992]) to make the dry weight soil concentrations comparable to wet weight concentrations in prey. The dietary fraction of incidental soil ingestion (2%) for GHOs was based on reports in the literature (USEPA 1993). An absorbance factor of 1.0 was applied to incidental soil ingestion exposures. Dioxin equivalent concentrations in soils were not measured at the site and were estimated from the Aroclor-based soil data using the methods described previously for waterfowl (Schwartz et al. 1993).

Comparisons of potential hazard estimated for dietary exposure to PCBs, were based on HQs. Hazard quotients were calculated as the APDD (ng PCB/g bw/d or pg

$TEQ_{WHO\text{-}Avian}$/g bw/d) divided by the corresponding TRV (Equation 2).

$$HQ = \frac{APDD(\text{ng PCBs/g bw/d or pg TEQs/g bw/d})}{\text{dietary TRV}} \quad (2)$$

Other lines of evidence from previously published studies on KRSS GHO populations were examined to minimize uncertainties in the analysis and calculation of potential risk from dietary exposure (Strause et al. 2007). These include the top-down risk assessment approach that quantified concentrations of PCBs present in GHO eggs and nestling plasma, and examined the effects of chlorinated hydrocarbons on egg viability through measurements of eggshell thickness and Ratcliffe index (Hickey and Anderson 1968; Ratcliffe 1968). A 3rd and ancillary line of evidence investigated potential effects of PCB exposures at the KRSS by monitoring productivity (fledgling success) and relative abundance between the contaminated floodplain habitat (TB) and the reference location (FC). By evaluating multiple lines of evidence together it was possible to provide the best available information for remedial decision-making at the site, especially when 2 or more lines of evidence converged on a common finding.

Statistical analysis

Both parametric and nonparametric statistics were applied depending on which assumptions were met. Concentrations of total PCBs and $TEQ_{WHO\text{-}Avian}$ in prey populations from the site were analyzed for normality by use of the Kolmogorov-Smirnov, 1-sample test with Lilliefors transformation. Concentrations of the COCs were generally log-normally distributed and therefore all data sets were log-transformed to more closely approximate the normal distribution. Data sets that were normally distributed were compared using a *t* test. If the data did not exhibit a normal distribution, then a nonparametric version of the *t* test (Mann–Whitney *U* test) was used. Associations between parameters were made with Pearson Product Correlations. Tests for normality and treatment effects (spatial trends) were completed using the Statistica (Version 6.1) statistical package (Statsoft, Tulsa, OK, USA). The criterion for significance used in all tests was $p < 0.05$. Statistical methods for comparing COC concentrations in GHO tissues (eggs, plasma), eggshell parameters (shell thickness, Ratcliffe Index) and call/response survey measurements of GHO abundance employed in the multiple-lines-of-evidence evaluation of site-specific risk to KRSS GHO populations have been described previously (Strause et al. 2007).

RESULTS

Composition of the diet of GHOs

A total of 285 discrete prey items were identified in 59 pellet and prey remains samples collected from a combined total of 7 active nests in the FC and TB study sites from 2000 to 2002. Excepting 4 post-fledge prey remains samples collected between 4 and 28 June, all samples were collected prior to 1 June in each year of the study, and, as such, the data provide a characterization of the spring or nesting season diet for KRSS GHOs. Only prey items represented by the classes *Aves* and *Mammalia* were observed. Prey from classes *Reptilia*, *Amphibia*, or *Crustacae* were not observed in the diets of GHO at the KRSS (Table 2).

Dietary compositions at FC and TB varied slightly when compiled on a class basis. Fort Custer GHOs consumed a slightly lesser proportion of birds and slightly greater proportion of mammals (birds: 15.5% numeric, 13.8% mass; mammals: 84.5% numeric, 86.2% mass) compared to TB GHOs (birds: 27.5% numeric, 24.8% mass; mammals: 72.5% numeric, 75.2% mass). Similar results are produced whether one compiles the class-level data on either a numeric or mass basis with only a slight increase in the proportion of mammalian prey when diet is compiled on a mass basis.

Within-class differences were observed between the FC and TB diets of GHO. Large differences were observed in the proportions of passerine/terrestrial birds represented in diets of GHOs at FC and TB (11% vs 25.8% on a numeric basis and 5% vs 22% on a mass basis, respectively) and in the proportion of rabbits represented in GHO diets at FC and TB (46% vs 16% on a numeric basis and 75% vs 50% on a mass basis, respectively). Within-class differences also are seen between diet compilations based on percent number versus percent biomass. On a numeric basis, small mammals (mice/voles) and shrews account for up to 33% and 51% of the GHO diet at FC and TB, respectively. These proportions decrease to 2.2% (FC) and 6.2% (TB) of the diet on a mass basis. Likewise, the combined proportion of rabbit and muskrat prey on a numeric basis increases from 51% (FC) and 21.5% (TB) to 84% (FC) and 69% (TB) on a mass basis.

A diet compilation based on mass provides the most accurate characterization of the relative importance of prey to avian predators (Marti 1987). Mass-based characterizations of KRSS GHO diet at FC and TB are compared to diet composition values for a nearby GHO subpopulation residing in southeast Michigan (Craighead and Craighead 1956; Figure 4). Class-level differences in prey composition between KRSS GHOs and the literature-based (LB) values are greatest for classes *Aves* and *Mammalia* at FC (13.8% [FC] vs 68% [LB], 86.2% [FC] vs 31.5% (LB), respectively). Crayfish (class *Crustacae*) were also present in the LB diet of GHO. Within-class prey proportions for KRSS and literature-based GHO diets were similar with passerine/terrestrial birds and rabbits/muskrats comprising the great majority of bird and mammalian prey.

Total PCB and $TEQ_{WHO\text{-}Avian}$ concentrations in prey

Concentrations of total PCBs and lipid content of 130 discrete whole-body prey item samples were determined. Prey items collected previously from the KRSS included 17 waterfowl samples that also were used to estimate GHO exposure to PCBs via the diet. Budget limitations prevented the collection and PCB/TEQ analyses of rabbit and grey/fox squirrel samples from FC and TB. Available data for chipmunk and red squirrel were used to fill this gap in the data base. Geometric mean concentrations of total PCBs in FC prey ranged from 2 ng PCBs/g ww in rabbit surrogates (i.e., chipmunk/red squirrel) to 9.6×10^1 ng PCBs/g ww in passerines (Table 3). Geometric mean concentrations of total PCBs in TB prey ranged from 5.6×10^1 ng PCBs/g ww in muskrats to 1.3×10^3 ng PCBs/g ww in passerines. Total PCB concentrations in waterfowl were 8.9×10^2 ng PCBs/g ww, and this value was used for both the FC and TB sites because of the uncertainty associated with residence and exposure of these mobile and migratory species. Polychlorinated biphenyl concentrations in prey items from TB were significantly greater (small mammals, muskrats, crayfish; *t* test $p < 0.01$;

Table 2. Great horned owl spring diet composition at the Kalamazoo River Superfund Site (site-specific) and from the literature

	Numeric basis (% occurrence)			Mass basis (% contribution)		
	Site-specific diet ($N = 285$)		Literature-based diet ($N = 260$)	Site-specific diet ($N = 285$)		Literature-based diet ($N = 260$)
Prey item	Ft. Custer (FC)	Trowbridge (TB)	Washtenaw Co. MI[a]	Ft. Custer (FC)	Trowbridge (TB)	Washtenaw Co. MI[a]
Class Aves	15.5	27.5	41.0	13.8	24.8	68.0
Passerine[b]	11.0	25.8	39.0	5.0	22.0	65.5
Waterfowl[c]	4.5	1.7	2.0	8.8	2.8	2.5
Class Mammalia	84.5	72.5	54.0	86.2	75.2	31.5
Mice/vole	31.0	49.0	41.0	2.0	6.0	2.5
Shrew	2.5	2.0	0.0	0.2	0.2	0.0
Muskrat	5.0	5.5	2.0	9.0	19.0	6.0
Rabbit[d]	46.0	16.0	11.0	75.0	50.0	23.0
Class Crustacae	0.0	0.0	5.0	0.0	0.0	0.5
Crayfish	0.0	0.0	5.0	0.0	0.0	0.5

[a] Craighead and Craighead (1956).
[b] Passerine category includes all terrestrial birds and all unidentified bird ("unknown bird") remains.
[c] Waterfowl category includes all aquatic birds.
[d] Rabbit category includes squirrels and all unidentified medium-size mammal ("unknown mammal") remains.

passerines, shrews; Mann–Whitney U test $p < 0.01$) than those from the upstream reference area at FC. Waterfowl and rabbit (surrogate) samples (TB sample size = 1) were not tested for significant differences.

Geometric mean concentrations of $TEQ_{WHO\text{-}Avian}$ in FC prey ranged from 0.52 pg TEQ/g ww in rabbit surrogates to 7.5 pg TEQ/g ww in crayfish (Table 4). Geometric mean concentrations of $TEQ_{WHO\text{-}Avian}$ in TB prey ranged from 1.3×10^1 pg TEQ/g ww in muskrats to 7.1×10^1 pg TEQ/g ww in rabbits. Using congener-specific fractional composition values (Schwartz et al. 1993) conservatively estimated $TEQ_{WHO\text{-}Avian}$ concentrations in 17 waterfowl samples were 2.4×10^2 pg TEQ/g ww. Prey items from TB contained significantly greater concentrations of TEQ than those from FC (t test $p < 0.02$) with the exception of muskrats and small mammals, which were not statistically different (Mann–Whitney U test $p = 0.26$ and $p = 0.22$, respectively). Waterfowl and rabbit (surrogate) samples (TB sample size = 1) were not tested for significant differences.

Contributions to total $TEQ_{WHO\text{-}Avian}$ from the 4 non-*ortho* and 8 mono-*ortho* PCB congeners showed that more than 90% of total TEQ was contributed by non-*ortho* congeners 77, 81, and 126 for all prey item categories at each of the 2 sampling locations excepting TB mice/voles (45% contribution; Figure 5). The same 3 coplanar congeners were among the 3 greatest contributors to concentrations of total TEQ for all prey item categories at each sampling location excepting TB mice/voles and TB rabbits (chipmunk). The 3 greatest PCB congener contributors and their combined contribution to total TEQ for each prey item category included: PCB 77 > 126 > 81 (FC passerine [95.4%], TB passerine [97%], FC and TB waterfowl [98.1% estimated], FC and TB crayfish [99%]; PCB 77 > 81 > 126 (TB muskrat [98.6%]); PCB 126 > 77 > 81 (FC muskrat [99.7%], FC rabbit [chipmunk and red squirrel] [99.1%], TB shrew [93.3%]); PCB 126 > 81 > 77 (FC shrew [99.4%], FC mice/vole [97.1%]); PCB 126 > 77 > 118 (TB rabbit [chipmunk] [97.9%]); PCB 105 > 81 > 126 (TB mice/vole [71.1%]).

Average potential daily dose

Average potential daily doses for GHOs were calculated based on geometric mean and upper 95% CL concentrations of both total PCBs and $TEQ_{WHO\text{-}Avian}$ of each prey item category for the numeric- and mass-based range of dietary composition at the FC and TB study sites (Table 5). Calculations of both total PCB and $TEQ_{WHO\text{-}Avian}$ exposures at TB included contributions from incidental soil ingestion. Based on site-specific diet and prey item COC concentrations, GHO ingestion of total PCBs were from 7- to 10-fold greater at TB than at FC, and $TEQs_{WHO\text{-}Avian}$ were 3-fold greater at TB than FC. Average potential daily doses calculated using the upper 95% CL (geometric mean) of total PCBs and $TEQ_{WHO\text{-}Avian}$ displayed a range of differences that were similar (6- to 7-fold difference and 2-fold difference, total PCBs, $TEQ_{WHO\text{-}Avian}$, respectively) to values of APDD based on the geometric mean.

Comparisons of geometric mean, mass-based ranges of APDD between the site-specific (APDD measured) and literature-based (APDD predicted) GHO dietary compositions yielded APDD values for total PCB exposures at FC that were equivalent (≤1.5-fold difference), and TB total PCB APDD values that differed by a factor of 1.6 (literature-based > site-specific APDD; Table 5). Average potential daily dose values for mean $TEQ_{WHO\text{-}Avian}$ were 1.6- to 2.3-fold greater for FC site-specific based dietary exposures and equivalent for TB based exposures. The literature-based TB APDD calculations for both total PCB and $TEQ_{WHO\text{-}Avian}$ included contributions from incidental soil ingestion consistent with the calculations for site-specific exposures at TB. Average

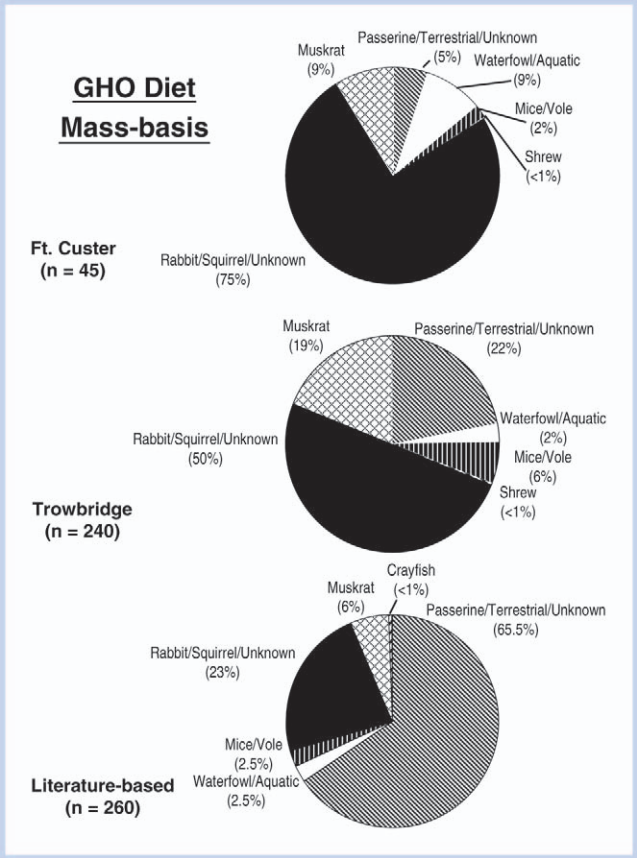

Figure 4. Site-specific great horned owl (GHO; *Bubo virginianus*) diet composition based on a biomass contribution basis for the Ft. Custer and Trowbridge sampling locations, and a literature-based (Craighead and Craighead 1956) diet composition for GHO populations in southeast Michigan (USA).

potential daily doses (site-specific vs literature-based) calculated using the upper 95% CL for total PCBs displayed a range of values similar to mean-based APDDs (FC exposures were equivalent, literature-based APDDs were 2.1-fold greater for TB exposures). Average potential daily doses calculated using the upper 95%CL concentrations of $TEQ_{WHO\text{-}Avian}$ in prey produced site-specific APDDs that were 1.5- to 2.1-fold greater than literature-based values at FC, and APDDs that were equivalent at TB.

The greatest calculated APDDs for GHOs at the KRSS originated from a mass-based dietary compilation. The 10-fold greater APDD at TB than at FC was consistent with the significant differences in total PCB and $TEQ_{WHO\text{-}Avian}$ concentrations in prey items collected from the 2 sites. The APDDs based on total PCBs and $TEQ_{WHO\text{-}Avian}$ for the site-specific and literature-based diets were less than 3-fold different for both FC and TB. The moderate differences in dietary composition observed at a class and within-class level between the 2 studies did not combine to influence APDD to a great extent.

Due to greater concentrations of COCs or greater proportions in the diet, exposures are often dependent on a few types of prey. This phenomenon was observed for GHOs at the KRSS where 2 to 4 prey item categories combine to account for more than 90% of the APDD at any given site and diet composition. At FC, mass-based APDDs for geometric mean total PCB concentrations in prey show that waterfowl (91% of APDD) and passerines (5%) drive exposures for the site-specific diet, and the same 2 prey items, albeit in an inverted ratio: waterfowl (25%) and passerines (72%), also drive the literature-based exposure (Figure 6A). At TB, passerines (45%), rabbits (43%), and soil ingestion (5%) figure predominantly in $APDD_{PCBs}$ for site-specific exposures and also literature-based exposures (passerines [62%], rabbits [26%], soil ingestion [5%]). The principal prey items responsible for APDDs calculated for $TEQ_{WHO\text{-}Avian}$ include the same prey identified for $APDD_{PCBs}$ but in some cases additional prey contribute to the 90% threshold. At FC, waterfowl (97%) and rabbits (2%) drive $APDD_{TEQ}$ for site-specific exposures, and literature-based exposures come from waterfowl (62%) and passerines (35%). At TB, rabbits (52%), passerines (18%), soil ingestion (16%), and waterfowl (10%) drive $APDD_{TEQ}$ for site-specific exposures, and literature-based exposures come from passerines (34%), rabbits (28%), waterfowl (16%), and soil ingestion (14%; Figure 6B).

Assessment of hazard

Hazard quotients were calculated for each location based on the site-specific and literature-based APDDs for total PCBs and $TEQ_{WHO\text{-}Avian}$. To conservatively estimate potential hazard to resident GHOs at the KRSS and to capture the broadest reasonable range of variability in characterizations of prey item COC concentrations and composition of the GHO diet, HQs are calculated from the range of APDD values encompassing the geometric mean and associated upper 95% CL values for each respective prey item and the dietary proportion contributed by each prey item compiled on both a numeric and mass basis. The range of HQs discussed for the NOAEL and LOAEL effect levels (HQ_{NOAEL}/HQ_{LOAEL}, respectively) will typically represent potential hazard associated with exposures to geometric mean concentrations for a numeric-based diet (low range) up to the upper 95% CL concentrations for a mass-based diet (high range). All HQs (total PCBs and $TEQ_{WHO\text{-}Avian}$) for site-specific and literature-based diets determined for both FC and TB geometric mean and upper 95% CL exposures were less than 1.0 (Table 6). The maximum FC HQ_{NOAEL} for total PCBs was 0.02 and 0.03 for the site-specific and literature-based diets, respectively. The maximum TB HQ_{NOAEL} for total PCBs was 0.15 and 0.31 for the site-specific and literature-based diets, respectively. The maximum FC HQ_{NOAEL} for $TEQ_{WHO\text{-}Avian}$ ranged from 0.27 to 0.13 for the site-specific and literature-based diets, respectively; TB $HQ_{NOAEL/TEQ}$ ranged from 0.5 to 0.73 for the site-specific and literature-based diets, respectively.

DISCUSSION

Dietary composition

The 3-y sampling program at active GHO nests for pellets and prey remains was an effective approach for characterizing site-specific exposures of nestling GHOs to the COCs at the KRSS, and to concurrently allow for nonintrusive monitoring of GHO productivity at each nest (Strause et al. 2007). Additionally, some potential biases commonly associated with pellet and prey remains sampling were also addressed in this study. Our approach capitalized on GHO preferences to use nests built by other bird species or in this instance artificial nesting platforms that were located at appropriate floodplain locations in the KRSS. In doing so, we successfully induced

Table 3. Geometric mean and upper 95% confidence level (U95% CL) concentrations of total polychlorinated biphenyls (PCBs) (ng PCBs/g wet weight) in prey items collected from 2 sites on the Kalamazoo River (MI, USA)

	Ft. Custer					Trowbridge				
	N	Range	Geometric mean	U95% CL	Lipid (%)	*N*	Range	Geometric mean	U95% CL	Lipid (%)
Passerines[a]	11	9–1,030	96	262	4.10	20	62–32,200	1,336*	3,102	4.90
Waterfowl[b]	17	130–28,000	889	1,751	3.80	17	130–28,000	889	1,751	3.80
Mice/Vole	12	2–180	13	27	3.59	20	30–548	67*	102	4.57
Shrew	16	2–18	8	10	3.66	17	25–3,150	847*	1,533	2.68
Muskrat	4	8–26	13	22	2.60	7	14–112	56*	94	2.07
Rabbit[c,d]	6	1–6	2	4	3.71	1	568	568	568	4.95
Crayfish	4	27–89	49	93	0.63	13	76–1,940	373*	597	1.62

* Trowbridge total PCB concentrations are significantly greater than concentrations at Ft. Custer ($p \leq 0.01$).
[a] Geometric mean total PCB concentrations in Ft. Custer house wren (5), tree swallow (2), and American robin (4) and Trowbridge starling (1), house wren (6), tree swallow (5), and American robin (8) used as representative of terrestrial passerine concentrations.
[b] Waterfowl samples were collected from 5 locations on the Kalamazoo River and were not divided between upstream and downstream sampling locations because of uncertain local residence status on the river.
[c] Geometric mean total PCB concentrations in Ft. Custer chipmunk (5) and red squirrel (1) used as surrogate value for rabbit concentration.
[d] Total PCB concentrations in Trowbridge chipmunk (1) used as surrogate value for rabbit concentration.

resident GHOs to occupy areas of the site having maximum exposure potential. Only one of the active nests sampled for pellets and prey remains was a natural nest, all remaining nesting activity included in the diet characterization study occurred at artificial nesting platforms. Additional advantages of GHO behavior incorporated into this study included their propensity to forage within relatively small areas because of their sedentary lifestyle and highly versatile prey capture ability (Marti 1974).

In this study, sampling of pellets and prey remains from beneath feeding perches and nest trees (ground collections) was augmented with collections from active nests during the blood sampling event and again after fledging was complete. Nest sites were the best locations for collecting avian prey remains, particularly feathers that could be positively classified as evidence of owl predation. Nest collections eliminated a significant source of uncertainty associated with feather remains collected on the ground beneath or in the general vicinity of active nests and feeding perches for which solid evidence of owl predation was mostly lacking.

Feeding studies with captive GHOs (Errington 1930; Glading et al. 1943) showed that pellets consistently reflected the food habits of adult and juvenile owls. Potential biases associated with pellet studies (under-representation of very

Table 4. Geometric mean and upper 95% confidence level (U95% CL) concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{Who\text{-}Avian}$) (pg $TEQ_{WHO\text{-}Avian}$/g wet weight) in prey items collected from 2 sites on the Kalamazoo River (MI, USA)

	Ft. Custer					Trowbridge				
	N	Range	Geometric mean	U95% CL	Lipid (%)	*N*	Range	Geometric mean	U95% CL	Lipid (%)
Passerine[a]	11	0.47–238	5.3	18	4.10	19	1.1–4,777	56*	194	4.90
Waterfowl[b]	17	36–7,678	244	725	3.80	17	36–7,678	244	725	3.80
Mice/vole	12	0.18–2.9	0.61	0.91	3.59	20	0.47–3.8	0.80	0.99	4.57
Shrew	16	0.45–49	1.28	2.18	3.66	17	4–249	47*	77	2.68
Muskrat	4	0.07–37	1.1	14	2.60	7	0.43–49	13	47	2.07
Rabbit[c,d]	6	0.13–4.0	0.52	1.51	3.71	1	71	71	71	4.95
Crayfish	4	1.5–58	7.5	33	0.63	13	3.1–374	56*	108	1.62

* Trowbridge TEQ concentrations are significantly greater than concentrations at Ft. Custer ($p \leq 0.02$).
[a] Geometric mean total $TEQ_{WHO\text{-}Avian}$ concentrations in Ft. Custer house wren (5), tree swallow (2), and American robin (4) and Trowbridge starling (1), house wren (6), tree swallow (5), and American robin (7) used as representative of terrestrial passerine concentrations.
[b] Waterfowl samples were collected from 5 locations on the Kalamazoo River and were not divided between upstream and downstream sampling locations because of uncertain local residence status on the river.
[c] Geometric mean total $TEQ_{WHO\text{-}Avian}$ concentrations in Ft. Custer chipmunk (5) and red squirrel (1) used as surrogate value for rabbit concentration.
[d] $TEQ_{WHO\text{-}Avian}$ concentrations in Trowbridge chipmunk (1) used as surrogate value for rabbit concentration.

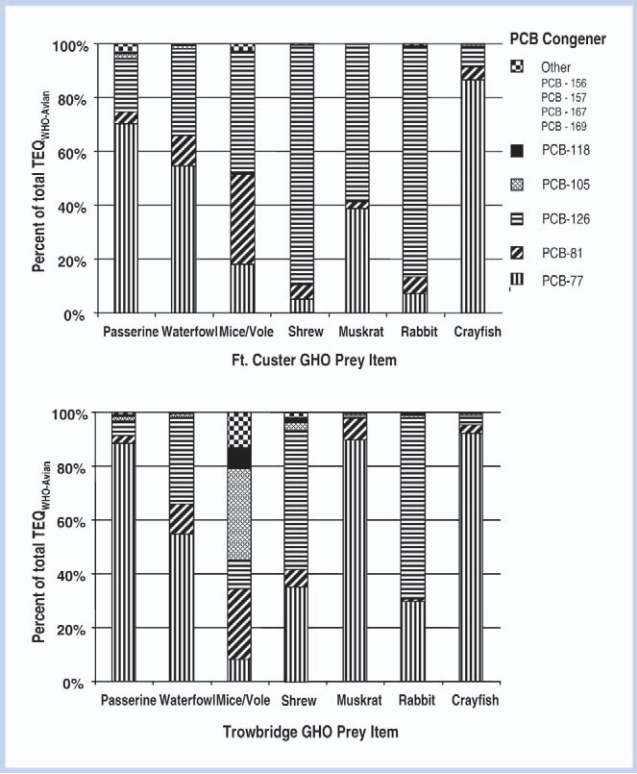

Figure 5. Percent contribution of polychlorinated biphenyl (PCB) coplanar and mono-*ortho*-substituted congeners to total 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) in great horned owl (*Bubo virginianus*) prey at the Kalamazoo River.

small prey, prey with more easily digestible components, boneless pellets from newly hatched owlets) or prey remains collections (over-representation of larger species and avian prey) can be adequately managed through collections of both types of samples, segregation of samples among active nests, and reconciliation of all concurrently collected samples. Very small prey items (e.g., very young animals) are unlikely to carry great COC concentrations because of a very limited period of potential exposure, and even in the exception (e.g., shrews, which do frequently have great COC concentrations), their low mass will contribute negligibly to APDD at most sites. Prey with easily digestible components may still be identified in prey remains collected from the nest, and the small numbers of prey that are completely absorbed by recently hatched owlets will most likely be adequately represented in subsequent pellets.

Studies of the dietary habits of North American GHO populations show the GHO is an opportunist hunter, plastic in foraging behavior, a generalist in prey selection with the broadest diet of any North American owl (Marti and Kochert 1996). Dietary preferences depend upon habitat, season, and prey vulnerability, and GHOs are capable of expressing the most diverse prey profile of all North American raptors (Voous 1998). Contributing factors to the species' broad diet include a large body size and crepuscular/nocturnal activity range. Major determinants upon prey selection by any individual include habitat, prey abundance, and prey vulnerability (a measure that combines ephemeral and interrelated determinants of prey density/prey behavioral patterns, habitat condition, and seasonality; Houston et al. 1998).

In general, temperate North American GHO populations feed predominantly on terrestrial mammals followed by terrestrial and aquatic birds, and a minor mix of reptiles, amphibians, and arthropods (Marti and Kochert 1995; Murphy 1997). Great horned owl diets vary between physiographic regions (Wink et al. 1987) and even among individual nesting territories when land use and habitat type distributions diverge within distinct physiographic units (Marti 1974). Great horned owl diets can also show significant temporal variation when pronounced changes in prey availability occur due to natural small mammal population cycles or anthropogenic modifications to habitat or prey populations (Fitch 1947; Adamcik et al. 1978). In addition, GHO diets also may vary with seasonal changes in prey vulnerability, although this source of variation tends to be minor compared to diet alterations stemming from

Table 5. Range of average potential daily doses (APDD)[a] based on geometric mean and the upper 95% confidence level (U95% CL) of prey items for total polychlorinated biphenyls (PCBs) (ng PCBs/g body weight [bw]/d) and 2,3,7,8 tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) (pg TEQ/g bw/d) when assuming 2 different dietary compositions for great horned owl (GHO) at the Kalamazoo River Superfund Site, Michigan, USA

	Ft. Custer		Trowbridge[b]	
	PCBs	TEQs	PCBs	TEQs
PCB-based dietary models				
Site-specific APDD (APDD measured)[c]				
Geometric mean	3–5	0.676–1.25	30–37	2.39–3.81
U95% CL	7–10	2.03–3.76	59–61	5.34–6.98
Literature-based APDD (APDD predicted)[d]				
Geometric mean	4–5	0.428–0.549	38–60	2.72–3.97
U95% CL	9–12	1.34–1.75	80–127	6.81–10.18

[a] The range of calculated APDD results from using diet estimations based on both total frequency (numeric basis) and biomass contribution (mass basis; see Table 2).
[b] Includes incidental ingestion of floodplain soils at the former Trowbridge impoundment.
[c] Based on results of field collected GHO pellets and prey remains from active nests at each Kalamazoo River study site.
[d] A study of GHO diet in Washtenaw County, Michigan, USA (Craighead and Craighead 1956).

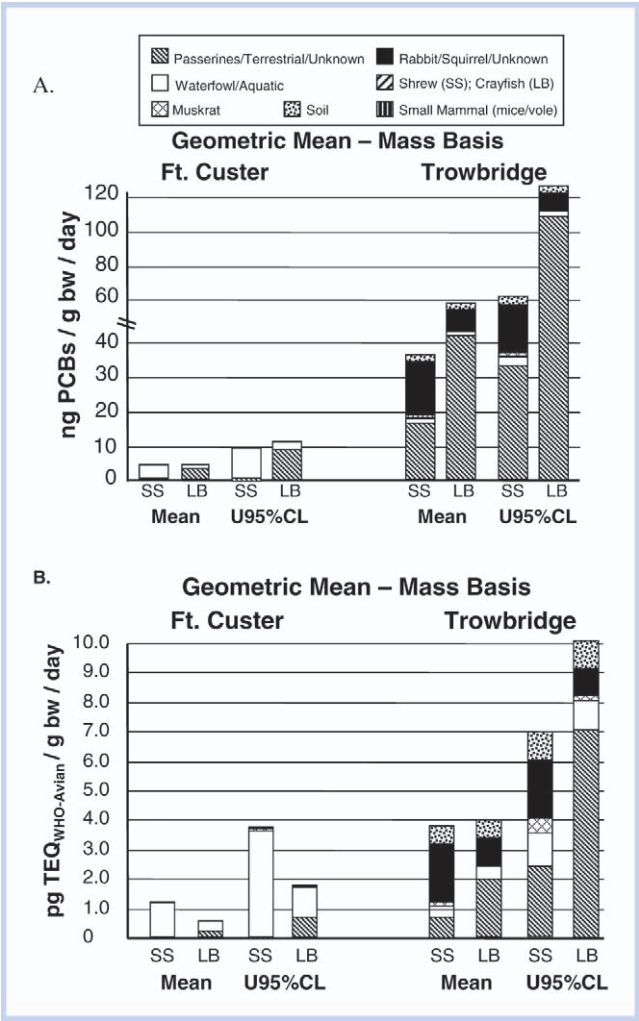

Figure 6. (**A**) Relative contribution of each principal prey component and incidental soil ingestion to total polychlorinated biphenyl (PCB) average potential daily dose (APDD; ng PCBs/g bw/d, ww) for great horned owl (*Bubo virginianus*) based on the geometric mean and upper 95% confidence level concentrations in Ft. Custer and Trowbridge prey, Trowbridge soil, and site-specific (SS) and literature-based (LB) dietary compositions (mass basis only). (**B**) Percent contribution of each principal prey component and incidental soil ingestion to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) average potential daily dose (APDD; pg TEQ/g bw/d, ww) for great horned owl (*B. virginianus*) based on the geometric mean and upper 95% confidence level concentrations in Ft. Custer and Trowbridge prey, Trowbridge soil, and site-specific (SS) and literature-based (LB) dietary compositions (mass basis only).

differences in habitat and temporal prey availability (Errington et al. 1940; Fitch 1947; Wink et al. 1987). If present, significant seasonal variations in GHO diets may originate from changes in prey vulnerability caused by a combination of factors including vegetation changes, altered activity patterns of prey and GHOs, day length, GHO reproductive cycles, prey hibernation patterns, prey migration patterns, and prey reproductive lifecycle events (e.g., mating, dispersal of young).

Great horned owl foraging preferences are difficult to predict from surveys of prey populations in most North American temperate habitats and attempts to correlate GHO predation preferences with prey abundances have yielded mixed results (Murphy 1997). This is due in part to the fact that prey density apparently has little effect on prey vulnerability in some GHO territories (Adamcik et al. 1978; Peterson 1979). Great horned owls tend to display a density-independent dietary relationship to prey species. Changes in prey vulnerability do not necessarily correspond with changes in numerical status. Some species remain more vulnerable to GHO predation regardless of annual fluxes in abundance/density, even in periods of low population densities (Errington 1932; Peterson 1979).

Attempts to correlate GHO predation preferences to habitat types show greater success where GHOs' food habits appear to depend largely upon where the bird is situated because many birds studied seem to limit their activities to a few acres of certain favorite habitat (Errington 1932; Fitch 1947). While there is evidence to support a strong interaction between GHO foraging preferences and habitat (Rusch et al. 1972), there were still instances where some GHOs did appear to respond opportunistically to the availability of certain prey types. (Marti and Kochert 1996). However, wetlands habitats appear to be a notable exception to the habitat–prey use relationship identified for GHOs where studies of GHO use of wetland-dependent prey and proximity and extent of wetlands within nesting territories have shown almost no relationship between habitat and prey type. In these studies, GHOs sought wetland prey regardless of proximity or abundance of wetland habitats. This may stem from the fact that prey species may be more available and vulnerable due to high prey density, high prey diversity and abundance, and more favorable locations and numbers of elevated hunting perches in wetlands and wetland edge habitats (Murphy 1977; Houston 1998).

Our studies of GHO predation at the KRSS included efforts to control for spatial and temporal variability in owl diets. Great horned owl nesting platforms were specifically located in riparian floodplain habitats that were buffered from most human disturbances and situated within 100 m of the river to provide uniformity in foraging habitat, available prey populations and habitat-dependent influences on prey vulnerability. Nest trees were selected after completing a qualitative survey of nesting habitat quality so as to provide an optimal mix of cover and foraging habitat for breeding owls. Pellets and prey remains from multiple years were collected only during active nesting and brooding periods of the annual reproductive lifecycle to provide uniformity in environmental cues on GHO behavior and seasonal influences on prey availability/vulnerability.

Site-specific and literature-based diets

Because the dominant factors influencing GHO diet originate from spatial differences in habitat type and temporal alterations in prey populations, a literature-based diet selected for use in the absence a site-specific value must match the physiographic region and dominant habitat types at the site. If multiple studies of equivalent quality are available, temporal considerations can also be addressed. For instance, in the 1940s, nesting habitat of GHO populations in southeastern Michigan (Superior Township, Washtenaw County) included plant and animal community assemblages that were very similar to those present at KRSS (Craighead and Craighead 1956). When dietary composition for GHOs from KRSS was compared to the Washtenaw study, differences were observed that were principally related to differing proportions of rabbits and passerine/terrestrial birds (Table 2). Kalamazoo River owls consumed greater proportions of rabbits, and Washtenaw County GHOs consumed greater proportions of

Table 6. Hazard quotient (HQ) values based on geometric mean and the upper 95% confidence level (U95% CL) of average potential daily doses (APDD) of total polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$), when assuming 2 different dietary compositions for great horned owl (GHO) at the Kalamazoo River Superfund Site, Michigan, USA[a]

	Ft. Custer		Trowbridge	
	HQ NOAEL	HQ LOAEL	HQ NOAEL	HQ LOAEL
PCB-based dietary models				
Site specific[b]				
Geometric mean	0.01	<0.01	0.07–0.09[c]	0.02–0.03
U95% CL	0.02	0.01	0.14–0.15	0.05
Literature based[d]				
Geometric mean	0.01	<0.01	0.09–0.15	0.03–0.05
U95% CL	0.02–0.03	0.01	0.20–0.31	0.07–0.10
$TEQ_{WHO\text{-}Avian}$ based dietary models				
Site specific[b]				
Geometric mean	0.05–0.09	0.01	0.17–0.27	0.02–0.03
U95% CL	0.15–0.27	0.01–0.03	0.38–0.50	0.04–0.05
Literature based[d]				
Geometric mean	0.03–0.04	<0.01	0.19–0.28	0.02–0.03
U95% CL	0.01–0.13	0.01	0.49–0.73	0.05–0.07

[a] Toxicity reference values used to calculate HQs are provided in Table 1. NOAEL = no observable adverse effect level; LOAEL = lowest observable adverse effect level.
[b] Based on results of field collected GHO pellets and prey remains from active nests at each Kalamazoo River study site.
[c] The range of calculated APDD results from using diet estimations based on both total frequency (numeric basis) and biomass contribution (mass basis; see Table 2).
[d] A study of GHO diet in Washtenaw County, Michigan, USA (Craighead and Craighead 1956).

passerines/terrestrial birds. The greater proportion of birds in Washtenaw County GHO diets is directly attributable to a greater number of ring-necked pheasants in the diet that were at their historical peak of abundance in Michigan during the 1940s. However, their number significantly decreased by the 1980s (Luukkonen 1998) and as a result, this loss of an important dietary item was compensated for by increased GHO consumption of cottontail rabbits in the KRSS study (Springer and Kirkley 1978; Peterson 1979).

The impact of wetlands on GHO dietary composition also needs to be taken into account in that wetland habitats are well represented and in close proximity to GHO nesting territories at KRSS and the proportion of species with a direct-link (habitat based; e.g., muskrat, waterfowl, crayfish) and indirect-link (foraging based; e.g., insectivorous passerine birds, bats, weasels) to the aquatic food-web may have an important contribution to the overall diet. The combined diet composition for KRSS GHOs (FC + TB) shows that 8.8% (numeric basis) and 21% (mass basis) of GHO prey originated wholly or in part from the aquatic food-web. In comparison, aquatic prey comprised 9% of Washtenaw County. A review of GHO diet studies available in the literature showed that aquatic prey are common in diets of GHOs residing in close proximity to wetland habitat types, and in select western and upper midwestern habitats, the proportion of aquatic prey (numeric basis) in resident GHO diets can exceed 20% and 50%, respectively (Murphy 1997; Bogiatto et al. 2003).

Average potential daily dose

A conservative approach was used to calculate APDD values for GHOs such that when site-specific PCB or $TEQ_{WHO\text{-}Avian}$ concentration data were not available for a specific component of GHO diet (e.g., rabbits, grey/fox squirrels), the shortcoming was addressed by using site-specific data for red squirrels and chipmunks to represent potential COC exposures for the group. This approach incorporated a conservative estimate of potential exposure because the omnivorous diets of squirrels and chipmunks place them in a higher trophic level compared to rabbits. The conservative nature of this substitution is evident in that the total PCB and $TEQ_{WHO\text{-}Avian}$ concentrations used to calculate rabbit contributions to Trowbridge APDD were 1 to 2 orders of magnitude higher than concentrations expressed by both terrestrial and aquatic herbivorous counterparts to rabbits at the site (mice/vole, muskrat; Table 3). A similar approach was used to address the absence of site-specific data for pheasants and other galliform prey where these species were grouped with passerine prey. Passerines at the site included insectivorous representatives (e.g., tree swallows) with forage-based links to the aquatic food-web at the site. Passerines had the highest concentration of total PCBs and 2nd highest concentration of $TEQ_{WHO\text{-}Avian}$ among prey groups from the TB site (Table 3). The US Fish and Wildlife Service waterfowl database also provided an unbiased characterization of potential exposure through this aquatic pathway by including

representative concentrations from both piscivorous (merganser) and omnivorous (mallard) feeding groups. Great horned owls prey indiscriminately upon waterfowl and a variety of wading birds (Rusch et al. 1972). Piscivorous waterfowl and shorebirds have been shown to accumulate total PCB and $TEQ_{WHO\text{-}Avian}$ concentrations that are 10- to 15-fold greater than their closely related avian counterparts who are more herbivorous (Jones et al. 1993). Processing of small mammals and avian prey that included the removal of stomach contents (both prey types) and feathers, beaks, wings, and legs (avian prey) is a common practice in exposure and effects studies and is typically used to conservatively estimate soil to organism bioaccumulation factors. The method contributed to conservative measurements of the COCs in these samples because the substantial mass excluded from analyses (keratin, herbaceous forage) contained much lower contaminant concentrations compared to the remaining tissues (predominantly muscle and lipids) analyzed for the target organism. This approach is also consistent with the consumption habits of GHOs which consume very small mammals in their entirety. Larger mammals are consumed in part by ripping the flesh from the skeleton and pelt. Birds are frequently plucked of their flight feathers and other larger contour feathers during or prior to being consumed. Finally, incidental soil ingestion was also included in the site-specific APDD calculations for TB exposures to account for soil that may be associated with the pelage of small mammalian prey that tunnel through vegetation or use burrows for shelter, nesting, or food storage, and also present in avian prey that consume grit and associated soil particles as a normal course of their foraging activities (Mayoh and Zach 1986).

A conservative approach also was used to estimate $TEQ_{WHO\text{-}Avian}$ from the PCB Aroclor data for waterfowl and soils in the calculation of $APDD_{TEQ}$ for GHOs. Dioxin equivalent concentrations ($TEQ_{WHO\text{-}Avian}$) were estimated by selecting the greatest proportional contribution of each individual non-*ortho* and mono-*ortho* PCB congener across 4 technical Aroclor mixtures as the fractional composition value for each medium. The greatest potential concentrations for each congener were supplemented with additional "enrichment factor" increases to 2 bioaccumulative and toxic non-*ortho* congeners (PCB 126 and 169). As a result, the estimated $TEQ_{WHO\text{-}Avian}$ concentrations for waterfowl and soils have much greater toxicity than would be predicted from the original Aroclor mixtures. Waterfowl $TEQ_{WHO\text{-}Avian}$ concentrations were the greatest among all prey type contributions to $APDD_{TEQ}$ by a factor of 3 and 7 for the geometric mean and upper 95% CL concentrations, respectively (Table 3).

The only notable difference between estimates of APDD from a site-specific diet ($APDD_{measured}$) and literature-based diet ($APDD_{predicted}$) were greater $APDD_{predicted}$ for total PCB (geometric mean and upper 95% CL values) at TB (mass-based diet), and greater $APDD_{measured}$ for $TEQ_{WHO\text{-}Avian}$ (geometric mean and upper 95% CL) values at FC (numeric- and mass-based diets). At TB, the greater $APDD_{PCB}$ values for the literature-based diet was due to the greater proportions of pheasants and to the elevated total PCB concentrations in the passerine/terrestrial avian prey group compared to total PCB concentrations in rabbits, the predominant prey group for TB owls. The difference in predicted versus measured $APDD_{PCB}$ was not observed at FC because the large differences in the proportion of passerine/terrestrial prey (e.g., pheasant) between the site-specific and literature-based diets was mitigated by the small total PCB concentrations in the dietary items collected at FC, a larger proportion of waterfowl in the FC $APDD_{measured}$ versus literature-based $APDD_{predicted}$, and the greater waterfowl total PCB concentrations used for the $APDD_{PCB}$ calculations (Tables 2 and 3; Figure 6). At FC, the greater $APDD_{TEQ}$ for the site-specific diet originated from the overriding influence of waterfowl prey. This included a larger proportion of waterfowl in the FC $APDD_{measured}$ versus literature-based $APDD_{predicted}$, and the much greater waterfowl $TEQ_{WHO\text{-}Avian}$ concentrations used for the $APDD_{TEQ}$ calculations (Tables 2 and 4; Figure 6). This difference in $APDD_{TEQ}$ was not present between $APDD_{predicted}$ /$APDD_{measured}$ at TB. The much larger proportion of passerine/terrestrial prey (e.g., pheasant) in the literature-based diet was mitigated by greater mean $TEQ_{WHO\text{-}Avian}$ in rabbits versus passerines/terrestrial avian prey, coupled with additional TEQ contributed from muskrat prey (with greater variable TEQ concentrations) to $APDD_{measured}$ that was calculated from the upper 95% CL for $TEQ_{WHO\text{-}Avian}$ in prey populations.

Overall, calculations of $APDD_{predicted}$ /$APDD_{measured}$ in this study were primarily influenced by gaps in the site-specific data for principal prey items in both the site-specific and literature-based diets. Although $APDD_{predicted}$ and $APDD_{measured}$ values were very similar across the range of prey concentration values at FC and TB, the notable differences observed in APDD can be traced to the lack of site-specific data for pheasants and rabbits, and the lack of recent, congener-specific total PCB data for waterfowl. Because all surrogate data used to address these data gaps was chosen to insure that any potential biases contributed by these data erred in a conservative "worst-case" manner, it is reasonable to assume that if site-specific data were available for these prey, the calculated $APDD_{PCB}/APDD_{TEQ}$ for both diets would have decreased and the relationships between $APDD_{measured}$ and $APDD_{predicted}$ for both total PCBs and $TEQ_{WHO\text{-}Avian}$ would have changed. This exercise also illustrates that differences between $APDD_{measured}$ and $APDD_{predicted}$ may be exacerbated at sites where the contaminant distribution between proximal aquatic and terrestrial habitats is dissimilar, and prey with links to the aquatic food-web figure predominantly in site-specific GHO diets. In these instances, the unique composition of a site-specific diet that includes aquatic prey may contribute significantly to the overall assessment of exposure, therefore posing significant potential risk that may be overlooked if the hazard assessment relies upon a literature-based dietary composition that fails to identify important prey items with links to aquatic exposures.

Hazard estimates based on total PCBs and TEQs

Hazard quotients based on $TEQ_{WHO\text{-}Avian}$ were greater than those based on total PCBs. Hazard quotients calculated from NOAEL TRVs for geometric mean and upper 95% CL concentrations of $TEQ_{WHO\text{-}Avian}$ were 4- to 13-fold greater than for total PCBs at FC, and 2- to 3-fold greater at TB (Table 6; Figure 7). Hazard quotients based on total PCB concentrations are considered to be an accurate estimate of potential risk because the concentration in the diet can be compared directly to values reported in the studies from which TRVs were derived. Congener-specific analyses provided for coplanar PCB congeners to be used in a calculation of TEQ. This

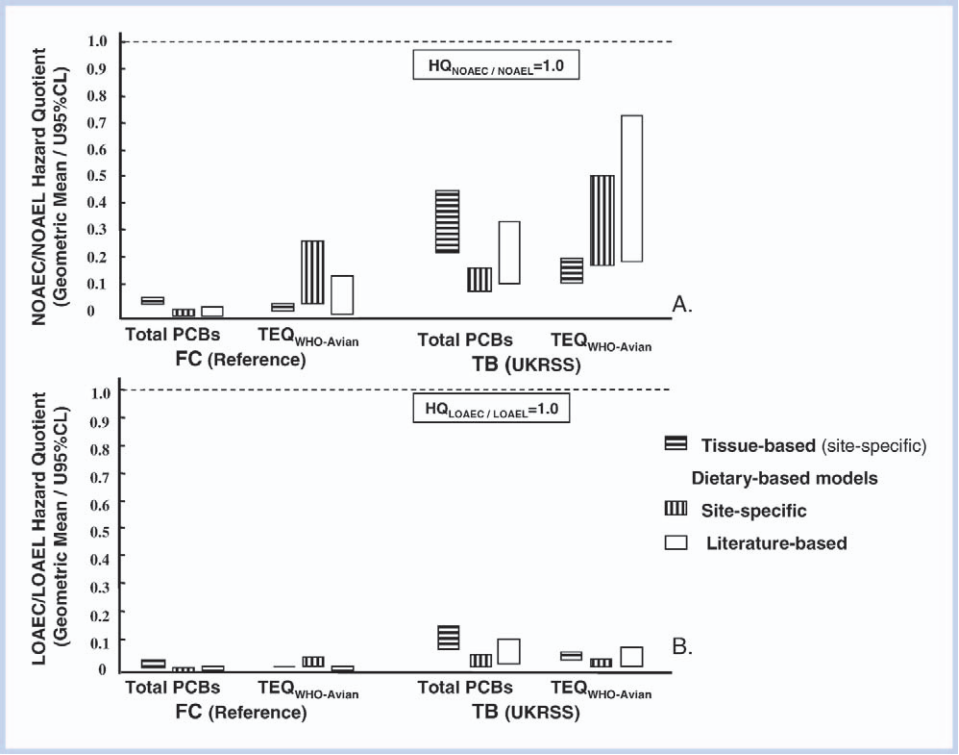

Figure 7. (**A**) Comparison of tissue-based (egg) no observable adverse effect concentration (NOAEC) and diet-based no observable adverse effect level (NOAEL) hazard quotients (HQs) at the Upper Kalamazoo River Superfund Site (Trowbridge) and Reference (Ft. Custer) locations calculated from NOAEC/NOAEL-based toxicity reference values (TRVs) for polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$). Each box encompasses the geometric mean and upper 95% confidence level concentration. Dietary HQ ranges include average potential daily dose (APDD) concentrations computed using numeric- and mass-based dietary compositions. (**B**) Comparison of tissue-based (egg) lowest observable adverse effect concentration (LOAEC) and diet-based lowest observable adverse effect level (LOAEL) Hazard Quotients (HQs) at the Upper Kalamazoo River Superfund Site (Trowbridge) and Reference (Ft. Custer) locations calculated from LOAEC/LOAEL-based TRVs for polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$). Each box encompasses the geometric mean and upper 95% confidence level concentration. Dietary HQ ranges include average potential daily dose (APDD) concentrations computed using frequency- and mass-based dietary compositions.

approach eliminated the difficulties and uncertainties involved with assessing the toxicity of environmentally weathered PCB mixtures that are quantified as Aroclors, and is generally believed to correlate better with toxicity than measures of total PCBs (Blankenship and Giesy 2002).

A number of factors contribute to the greater $TEQ_{WHO\text{-}Avian}$ HQ values calculated in this study. The absence of a well-designed TEQ feeding study for *Strigiformes* (or a more closely related raptor species) introduced uncertainties to the TRV screening process (discussed previously) and necessitated selection of a conservative TRV for HQ_{TEQ} calculations. The scientific basis for TEQ derivation and use may contribute to bias that overestimates risk when TEQs are applied to complex mixtures of PCBs. Concentrations of TEQs are calculated by multiplying each PCB congener by a class-specific (mammal, bird, fish) relative potency expressed as a toxic equivalency factor. Toxic equivalency factors are consensus values that were rounded up to be conservative estimates of potential risk (Van den Berg et al. 1998). These practices, coupled with the use of proxy values for congeners that were present at concentrations less than the method detection limit (e.g., use of one-half the method detection limit for nondetects), the summation of co-eluting congeners into a single value for some mono-*ortho* PCB congeners, and conservative estimates of congener fractional composition values for historical Aroclor PCB data are likely reasons that HQs based on $TEQ_{WHO\text{-}Avian}$ are greater than HQs estimated for total PCBs. Additionally, a recent review of tree swallow exposure studies indicated that $TEQs_{WHO\text{-}Avian}$ calculated from field-based TCDD and PCB exposures did not elicit similar endpoints of effect and may not be toxicologically equivalent (Neigh, Zwiernik, Blankenship, et al. 2006).

The multiple-lines-of-evidence approach

This study has determined that dietary exposures of resident GHO populations to total PCBs and $TEQ_{WHO\text{-}Avian}$ present in contaminated floodplain soil of the KRSS are well below the threshold for effects on reproductive success. Even when the most conservative estimates of HQ are considered in the bottom-up assessment of potential hazards at the site, all HQ values for calculated APDDs using a site-specific dietary composition are less than 0.5. Similarly, all HQ values for calculated APDDs using a literature-based dietary composition at the site are less than 0.75 (Table 6). The greater HQ value for the literature-based diet originated from overestimates of the passerine/terrestrial proportion of avian prey.

The bottom-up assessment was one component of a multiple-lines-of-evidence approach that also included a tissue-based top-down investigation of GHO exposure by investigating PCB concentrations in eggs and nestling plasma (Strause et al. 2007). Results of the tissue-based studies were consistent with the dietary findings (Figure 7). The observed total $PCB/TEQ_{WHO\text{-}Avian}$ concentrations in eggs resulted in HQs less than 1.0 for all exposures indicating that tissue-

based exposures did not pose a significant potential risk to GHO populations at the Upper KRSS. Hazard quotients calculated for tissue-based and site-specific dietary exposures show strong agreement at both the Reference and Upper KRSS study sites with less than a 3-fold difference between the ranges of $HQ_{NOAEC/NOAEL}$ and $HQ_{LOAEC/LOAEL}$ (mean and upper 95% CL concentrations) for both COCs, excepting $TEQ_{WHO\text{-}Avian}$ concentrations at FC where a 7-fold range in HQs was present.

The multiple-lines-of-evidence approach included ancillary investigations to the top-down assessment that focused on evaluating the relative abundance, site use, and productivity of resident GHOs at the Upper KRSS relative to the upstream Reference location (Figure 1). The relative abundance of territory-holding nesting pairs of GHOs in the Upper KRSS was near the carrying capacity for the available habitat area included in the study. Nest acceptance rates and nest fidelity of actively breeding Upper KRSS GHOs across all nesting seasons included in the study were consistent with previous studies of artificial nest acceptance and habitat usage by Strigiforms in midwestern forests (Holt 1996). Mean productivity rates (fledglings/active nest) were similar among locations where exposures to PCBs were much different, and were consistent with productivity measures for healthy midwestern GHO populations (Holt 1996; Strause et al. 2007). These results agree with findings for both the top-down and bottom-up approaches to evaluate chemical exposures at the site, and serve to reduce the uncertainties associated with assessment endpoints and strengthen the conclusion that potential risk to GHOs from exposures to total PCB/ $TEQs_{WHO\text{-}Avian}$ in the Upper KRSS are unlikely to be sufficient to cause adverse effects.

Results from this study suggest that it would be appropriate to estimate potential risk based on either tissue-based or dietary-based methodologies. However, if a dietary-based approach to estimate potential risk to GHOs is used, studies of site-specific diet must be completed to assure that site-specific data can be collected for principal prey items representing potential exposures for both aquatic and terrestrial food webs at any site where aquatic habitats are located in close proximity to resident GHO nesting habitats. Additionally, because budget limitations will constrain the breadth of prey item sampling and analyses at most sites, it is essential that risk assessors clearly communicate all dietary assumptions applied to the data (e.g., prey groupings, gaps in the chemical database for any prey comprising a denotive dietary proportion) and how these assumptions impact risk calculations at the site.

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